The Influence of PRP on Early Bone Formation in Membrane Protected Defects. A Histological and Histomorphometric Study in the Rabbit Calvaria

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ABSTRACT

Background: Platelet rich plasma (PRP) has been proposed to be a useful adjunct to bone grafting.

Purpose: The aim of the present study was to assess new bone formation in bone regeneration procedures using platelet rich plasma (PRP) alone or in combination with autogenous bone.

Materials and Methods: Four surgically created, monocortical defects 5 mm in diameter in the calvariae of 15 New Zealand rabbits were grafted with a coagulum-filled control, PRP, particulated autogenous bone alone (A), or combined with PRP (A-PRP).

Results: Mean platelet concentration of 1,761,930 ± 680,200/μl was achieved (5.30 ± 2.63 × fold of baseline). Animals were sacrificed 1, 2, and 4 weeks later. Histomorphometric analysis showed no statistical difference for total new bone formation at any time point, however, a detailed analysis revealed a statistically significant higher percentage of lamellar bone than woven bone for the autogenous bone group at 2 weeks; all other groups demonstrated equal percentages of either bone type. At 4 weeks, all groups revealed a statistically greater component of lamellar bone over woven bone. Graft resorption rate was similar for both A and A-PRP. PRP platelet concentration was significantly positively correlated with TGF-beta1 but not with PDGF-AB.

Conclusions: Within the limits of the chosen animal model, this study demonstrated that PRP during early healing, whether alone or mixed with autogenous bone, did not lead to greater bone remodelling, as compared to coagulum. In contrast, autogenous bone alone demonstrated accelerated bone remodelling at 2 weeks.

KEY WORDS: autogenous bone, bone graft, bone healing, bone regeneration, calvarial bone defect, platelet-rich plasma

INTRODUCTION

Autogenous bone is still considered to be the gold standard in grafting procedures as it contains not only osteoconductive but also osteoinductive and osteogenic properties. However, the conventional disadvantages of an additional surgical site, increased postoperative morbidity, increased surgical time, and limited supply remain reasons for searching for alternative sources of grafting material.[1]

An autologous preparation of platelets in a smaller than usual volume of plasma, platelet-rich plasma (PRP) has been the focus of many bone regeneration studies. The effect of PRP is based on the concept that a four or five times the basal concentration of autologous platelets (150,000–450,000/μl) releases significant quantities of

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DOI 10.1111/j.1708-8208.2009.00266.x

platelet growth factors which promote bone healing.[2] Because of the relatively short life span of platelets within the wound and subsequently the influence of its growth factors,[2] the peak effect of PRP would be expected to occur in the early stage of bone healing. Platelets contain various growth factors involved in initial wound healing: three isomers of platelet derived growth factor, PDGF-AB(-AA, -BB, and -AB), transforming growth factors-β (TGF-beta) (-β1 and -β2), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and epithelial-cell growth factor (ECGF).[2] These growth factors are released through platelet activation with bovine thrombin or calcium chloride. In addition, PRP is known to contain cell adhesion molecules such as fibrin, fibronectin, vitronectin, which participate in osteoconduction and epithelial cell migration. These components found in PRP are thought to promote bone regeneration and increase vascularity during wound healing.[3] PRP in combination with autogenous bone has been found to accelerate graft maturation and leads to a denser spongiosa following bone regeneration procedures of mandibular discontinuity defects after 6 months.[4]

Although conceptually compelling, PRP generates contradicting discussions with respect to its effects in vivo. In spite of some positive or even significant effects with the addition of PRP to various grafting materials in various bone regeneration procedures,[5–9] some studies could not demonstrate significant differences beyond early healing,[10–12] while other studies even revealed *impaired* bone formation with the addition of PRP.[13] Little has been clarified regarding the effect upon graft resorption or the maturation of the newly formed bone. In the present study, the effects during initial phases of bone healing were compared among the following groups: coagulum-filled control, PRP alone, particulated autogenous bone with and without PRP. Total new bone formation, as well as the individual components of bone formation, osteoid, woven, and lamellar bone, were evaluated. Effect upon graft resorption was studied. Additionally, correlations of platelet concentration and growth factors, TGF-beta1 and PDGF-AB, were analyzed.

MATERIALS AND METHODS

Study Design

The study protocol was approved by the Committee for Animal Research, State of Berne, Switzerland (approval no. 112/02). The experimental study was conducted in 15 adult New Zealand rabbits, each weighing between 3.2 and 4 kg (mean weight: 3.7 kg [S.D. 0.3 kg]).

Three groups each containing five animals were used to analyze three different healing periods of 1, 2, and 4 weeks. The healing periods were selected based on observations of a previous study, in which investigators demonstrated a trend of increased bone formation by the supplement of PRP to autogenous bone grafts at 1, 2, and 4 months in rabbit calvariae[10] and suggested that peak efficacy of PRP could have been observed at earlier.

Anesthesia and Animal Care

The animals were premedicated with ketamine, 25 mg/kg (Narketan®, Vétoquinol, Bern, Switzerland) and 0.25 mg/kg of medetomidine (Domitor®, Pfizer, Zürich, Switzerland) intramuscularly. Following intubation, general anesthesia was induced by 1.5–1–75% isoflurane (Attane™, Provet AG, Lyssach b. Burgdorf, Switzerland). Postoperatively, the animals were given antibiotic treatment with Clamoxyl® (amoxycillin, SmithKline Beecham AG, Thörishaus, Switzerland) for 5 days and analgesics for 3 days (Novalgin®, Aventis, Zurich, Switzerland; 50 mg/kg, once a day intramuscularly). All animals were checked daily for the duration of the observation period.

Surgical Protocol

Following a midline incision on top of the head, the vault of the skull was exposed. Four circular bony defects (diameter 5 mm, depth 1.5 mm) were drilled with a trephine into the outer cortex (*tabula externa*), two on each side of the sagittal suture approximately 1 cm apart anteriorly-posteriorly. Outer cortex blocks and the inner cancellous portion were removed, leaving the inner cortex (*tabula interna*) as the base of the defect. The corticocancellous blocks were ground to a particulate size of 1.0–1.5 mm using a bone mill (Schlumbohm, Brokstedt, Germany). Additional cancellous bone chips (approximately 400–800 μm) were harvested from the floor of the defects using a small chisel and mixed with the particulated autogenous bone for a final ratio of 1:1 between cortical and cancellous bone. The four defects were grafted with either: (1) coagulum-filled control (C), (2) particulated autogenous bone control (A), (3) PRP alone, or (4) PRP mixed with particulated autogenous bone (A-PRP). Treatment was assigned randomly but always paired anteriorly or

posteriorly based on the presence of PRP to avoid potential cross-contamination.

Approximately 5.0 mL of PRP was prepared immediately prior to surgery according to standard protocol (Harvest Technologies Corporation, Plymouth, MA, USA) but with a reduction in volume because of animal size. From the external jugular vein, 38 mL of blood was drawn into a syringe containing 4.3 mL of ACD-A anticoagulant (Grosse Apotheke, Interlaken, Switzerland) for overall 10% dilution. Using a dual applicator, PRP was consistently combined at a ratio of 10:1 with a 10% $CaCl_2$ (Monico S.P.A., Venezia/Mestre, Italy) /topical bovine thrombin (Thrombin-JMI®, GenTrac, Inc., Middleton, WI, USA) mixture (5 ml 10% $CaCl_2$ with 5,000 units of topical bovine thrombin), as indicated by the manufacturer, for growth factor release. In one region (anterior or posterior), PRP alone (0.5 ml) and PRP (0.5 ml)/autogenous bone mixtures were introduced to the two bone defects and served as test sites. The remaining two defects were filled with coagulum and autogenous bone. All defects were covered individually with a resorbable, porcine-derived, collagen membrane for filler stability (Bio-Gide®, Geistlich Biomaterials, Switzerland). Wound closure was achieved in a two-layer technique (Gore-Tex® CV-5, W.L. Gore & Associates, Inc., Flagstaff, AZ, USA and Vicryl® 5-0, Ethicon, Johnson & Johnson, Brussels, Belgium).

Sacrifice

Following premedication as used presurgically, the animals were euthanized with Nembutal®, 1.4 ml/kg (Abbott Laboratories, North Chicago, IL, USA). The harvested block specimens were immediately immersed in a solution of 4% formaldehyde and 1% calcium for transport.

Histological Preparation

Specimens were fixed in 10% neutral buffered formalin combined with 1% $CaCl_2$ for 2 weeks, whereafter each specimen was dehydrated and embedded in methylmethacrylate. Using a slow-speed diamond saw (Varicut VC-50, Leco, Munich, Germany) with copious irrigation, each specimen was sectioned transversally, yielding nondecalcified 500-µm-thick sections. The sections were mounted onto opaque Plexiglass with acrylic glue and ground to a final thickness of 80–100 µm and surface stained with toluidine blue/McNeal and basic fuchsin.[14]

Hematological Analysis and Growth Factor Quantification

Complete blood count and platelet counts were performed on samples of whole blood, PRP, and platelet-poor plasma (PPP) to demonstrate the effectiveness of the platelet concentration procedure.

Additional PRP samples were stored in Eppendorf tubes at minus 78°C[15] until quantification. Samples were then thawed and centrifuged for 10 minutes at 10,000 rpm immediately prior to assay at room temperature. Each sample was analyzed for PDGF-AB and TGF-beta1 using the Quantikine ELISA kit DHD00B and DB100B, respectively (R&D Systems, MN, USA).[15]

Histomorphometric Evaluation

The sections of each defect were histomorphometrically analyzed in duplicate by experienced examiners (N.B. and B.H.) who were blinded to the treatment modalities. The defect borders were delineated by the inner cortex, the surgically created lateral bone walls, and the membrane.

In the sections, the amount (%) of newly formed bone and its individual components (osteoid, woven, and lamellar bone), residual autogenous graft (where applicable), and soft tissue/marrow space were determined by point counting employing a square grid (distance between 6×6 test lines: 255 µm) at a magnification of ×6.3[16], utilizing a systematic random sampling protocol.[17]

Statistical Analysis

The membrane was either slightly expanded with the harder filler types (autogenous bone or autogenous bone with PRP) or slightly depressed with the softer filler types (coagulum or PRP alone). To exclude the possibility of a confounding effect, the data were initially expressed in duplicate for statistical analysis with the total area dictated by the membrane or original external cortex, respectively.

The initial descriptive analysis of the data was done using scatter plots for the distribution of the analyzed tissues (bone, bone classes as osteoid, woven or lamellar bone, and graft material). Potential differences between the different treatment types (C, PRP, A, and A-PRP) were evaluated for each time point (Kruskal–Wallis tests). Differences between woven and lamellar bone percentages for each treatment type at the 2 and 4 weeks (Wilcoxon signed rank tests) as well as differences in

residual graft over time (Kruskal–Wallis tests) and between A and A-PRP at each different time point were evaluated (Wilcoxon rank sum tests).

Correlation between the two different data sets (membrane- and cortex-expressed) were calculated (Pearson and Spearman correlation coefficients). Possible correlation were evaluated between the platelet concentration in PRP and the concentration of either TGF-beta1, PDGF-AB, or the WBC count in PRP. Additionally, the correlation between the WBC in PRP and the concentration of TGF-beta1 or PDGF-AB in PRP were calculated. Due to the small sample size in this study, the *p* values were not adjusted for multiple testing. The analysis was performed using the SAS Enterprise Guide 4.1 (based on SAS 9.1, SAS Institute Inc., Cary, NC, USA). Significance was considered $p < .05$.

Since no statistically significant differences were found between the membrane- and the cortex-expressed data sets and all data parameters of new bone formation revealed a high linear correlation (0.9833 and 0.9823, for Pearson and Spearman correlation coefficients, respectively), both data sets could be essentially regarded as equal. For simplicity, the authors opted to display only one data set. The membrane-expressed data were chosen specifically not only for convention but also because the membrane could always be identified, and therefore was an actual and not theoretical border. Most importantly, it reflected the guided bone regeneration principle of maintaining a surgically created space at a bony defect via a barrier membrane.[18]

RESULTS

Postoperative Evaluation

All animals healed uneventfully.

Laboratory Analysis of PRP

The PRP procedure yielded a mean platelet concentrate that was 5.30 ± 2.63-fold higher than the average platelet count in whole blood (Table 1). The mean PRP platelet concentration was 1,761,930 ± 680,200/μL while the mean whole blood platelet concentration was 362,730 ± 132,070/μL. The mean growth factor concentrations were quite variable: for PDGF-AB, 340.14 ± 240.77 pg/mL and for TGF-beta1, 56,539.84 ± 30,985.35 pg/mL. There was little correlation between the platelet concentration in PRP and the growth factor concentration of PDGF-AB and the white blood cell (WBC) count in PRP (Table 2). Only the platelet concentration in PRP and TGF-beta1 in PRP exhibited a statistically significant correlation. The correlation between the WBC in PRP and PDGF-AB concentration was also low, even slightly negative. The correlation

TABLE 1 Laboratory Values

Sample	Mean	SD	Range	Unit
PRP				
White blood cell count (WBC)	13.42	7.68	0.9–26.56	($\times 10^9$/L)
Hematacrit (HCT)	0.05	0.03	0.01–0.15	(L/L)
Platelet (PLT)	1,761.93	680.20	849–3028	($\times 10^9$/L)
PDGF-AB concentration	340.14	240.77	31.20–910.60	(pg/mL)
TGF-β1	56,539.84	30,985.35	15,126.14–115,411.22	(pg/mL)
Whole blood				
WBC	4.77	1.23	2.89–8.18	($\times 10^9$/L)
HCT	0.30	0.02	0.27–0.33	(L/L)
PLT	362.73	132.07	138–619	($\times 10^9$/L)
PPP				
WBC	0.03	0.02	0.01–0.08	($\times 10^9$/L)
HCT	0.0001	0.0004	0–0.001	(L/L)
PLT	40.00	11.67	20–70	($\times 10^9$/L)
Platelet Concentration				
PLT concentration (PRP) : PLT concentration (whole blood)	5.3	2.63	2.62–13.74	

PPP = platelet-poor plasma; PRP = platelet-rich plasma.

TABLE 2 Correlation between the Platelet Concentration in Platelet-Rich Plasma (PRP) and the Concentrations of TGF-β1, PDGF-AB, and the White Blood Count (WBC) in PRP; and the Correlation between WBC in PRP and the Concentrations of PDGF-AB and TGF-β1 Using Pearson and Spearman Correlation Coefficients

Correlation	Pearson	Spearman
PLT_PRP : TGF-β_PRP	0.52186*	0.59643*
PLT_PRP : PDGF-AB_PRP	−0.33306	−0.28061
PLT_PRP : WBC_PRP	0.26711	0.36429
WBC_PRP : PDGF-AB_PRP	−0.11453	−0.16443
WBC_PRP : TGF-β1_PRP	0.56886*	0.55714*

*$p < .05$.

between the WBC in PRP and TGF-beta1 concentration was statistically significant.

Histology

One Week. Figure 1 shows representative micrographs for the coagulum (Figure 1, A), the PRP (Figure 1, B), the autogenous bone (Figure 1, C), and the autogenous bone + PRP (Figure 1, D) groups. For all treatment modalities, the borders of the defect were clearly defined. The base of the defect was preserved as a solid bone plate that represented the original *lamina interna.* The collagen membrane remained as a straight line connecting the lateral defect walls without a significant collapse into the defect from lack of filler support through the coagulum (Figure 1, A) or the PRP (Figure 1, B). For the autogenous bone groups without (Figure 1, C) and with the PRP (Figure 1, D), the collagen membrane was level or slightly convex. For all treatment groups, the defect area was occupied with residual coagulum that was infiltrated with a granulation tissue and a provisional matrix, part of which consisted of woven bone lined by an osteoid seam and osteoblasts, indicating ongoing bone formation (Figure 2, D). The intertrabecular space was filled with a tissue resembling immature (primary) bone marrow. For the autogenous bone groups without (Figure 1, C) and with the PRP (Figure 1, D), the autogenous bone particles were clearly seen dispersed in the defect area. In contrast to the autogenous bone group without PRP (Figure 1, C), the central defect area of the autogenous bone group

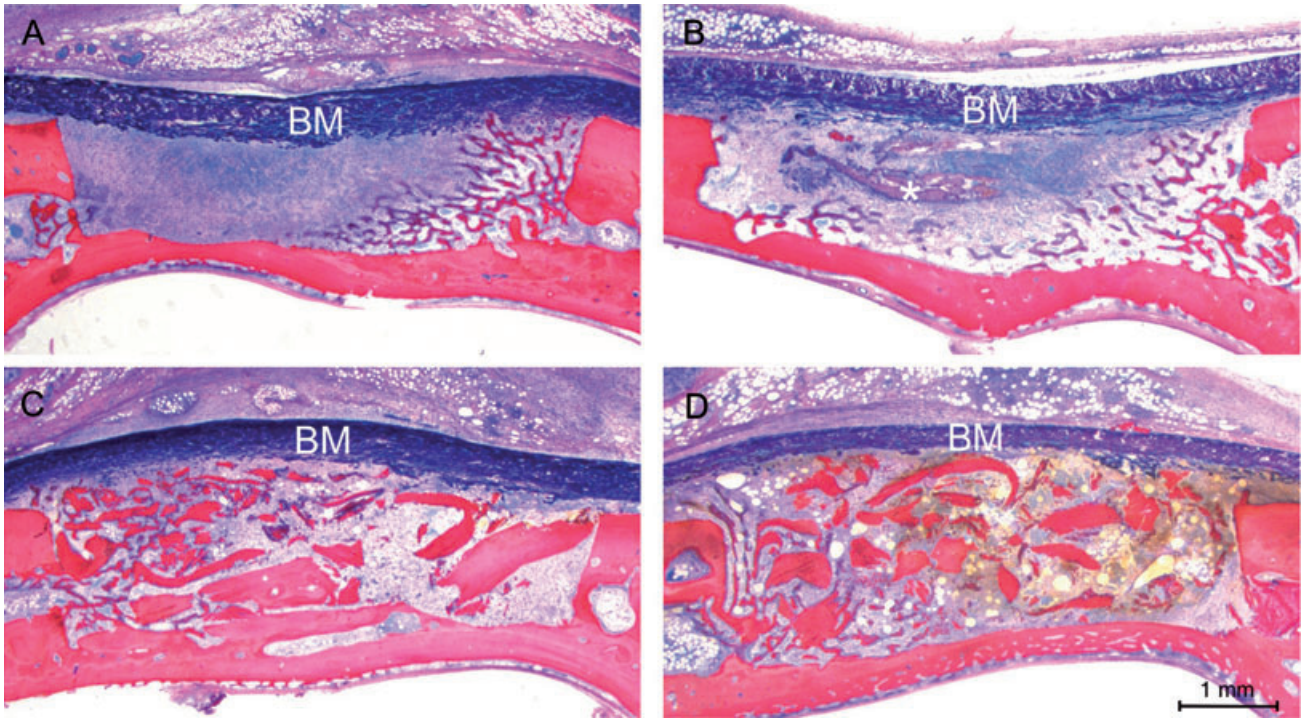

Figure 1 Overview micrographs of defects grafted with *A*, coagulum; *B*, PRP; *C*, particulated autogenous bone; and *D*, PRP mixed with particulated autogenous bone after 1 week. Each bone defect is lined by three bone walls (one at the defect base and two lateral to the defect) and a collagen barrier membrane (BM). The autogenous bone particles are clearly seen dispersed in the defect areas. Woven bone formation into the defect areas begins from the bone defect walls. Note the PRP concentration (*asterisk*) in the center of the bone defect in the PRP group.

with PRP remained occupied by PRP (Figure 1, D). However, most of the PRP was seen concentrated in the center of the defect in the PRP group (Figure 2, C).

For all treatment groups, the outgrowth of the woven bone trabeculae began along the bone defect walls (Figure 2, A). In the autogenous bone groups without (Figure 1, C) and with the PRP (Figure 1, D), some autogenous bone particles close to the defect walls were covered with newly formed woven bone, creating an occasional bridging between bone graft particles and the defect walls (Figure 2, B). On the majority of the autogenous bone graft particles (particularly away from the bone defect borders), however, bone deposition was not yet seen. Instead, some osteoclasts were observed to populate some of the autogenous bone graft particles.

Two Weeks. Figure 3 shows representative micrographs for the coagulum (Figure 3, A), the PRP (Figure 3, B), the autogenous bone (Figure 3, C), and the autogenous bone + PRP (Figure 3, D) groups. While the collagen membranes in the coagulum (Figure 3, A) and PRP (Figure 3, B) groups were slightly concave, the autogenous bone group without (Figure 3, C) and with PRP (Figure 3, D) showed a slightly convex or level collagen membrane, respectively. For all treatment modalities, newly formed bone had bridged the entire defect area under the collagen membrane with a scaffold of woven bone. With respect to the autogenous bone groups without (Figure 3, C) and with PRP (Figure 3, D), all graft particles were covered with new bone, lined by an osteoid seam and osteoblasts, and were thus integrated

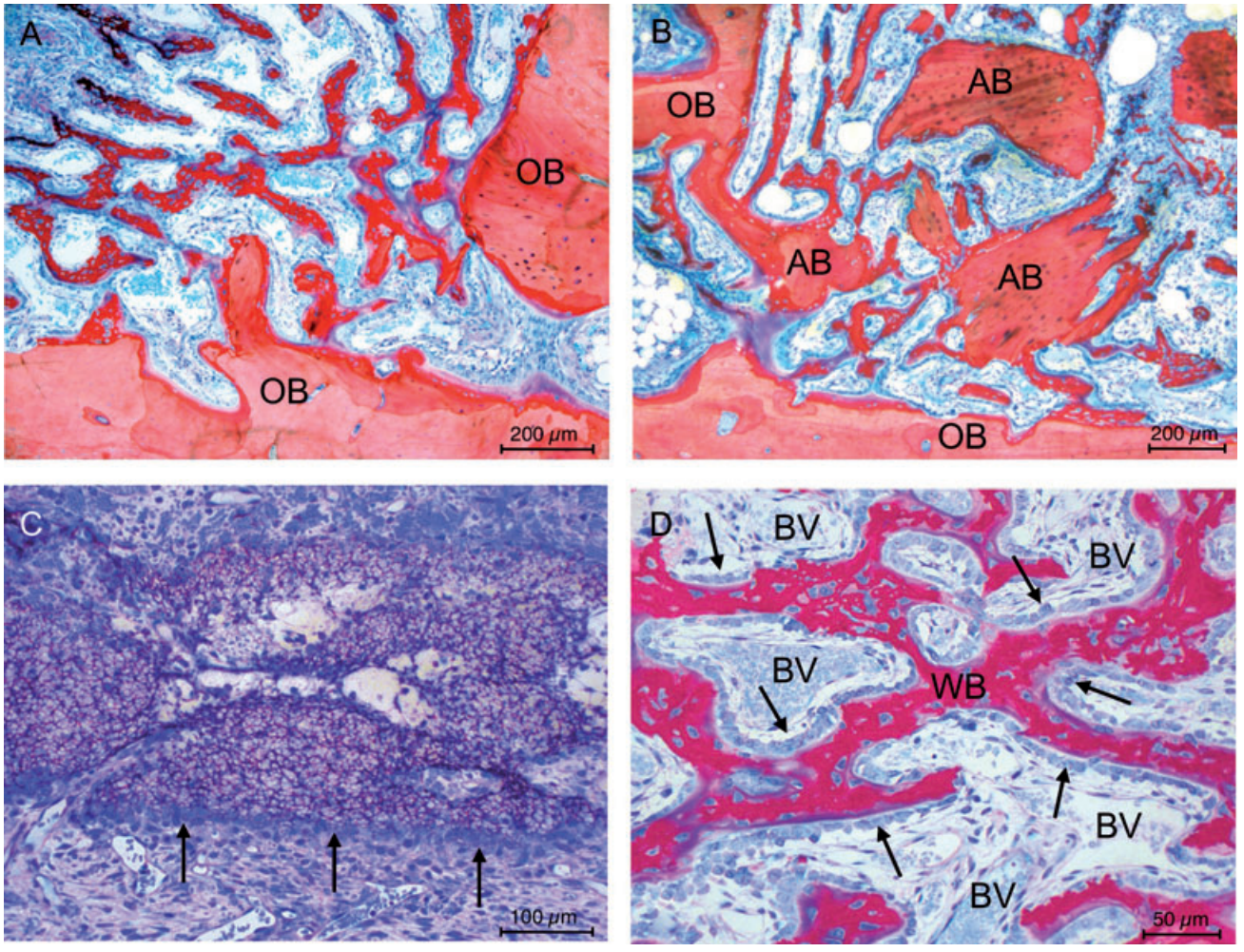

Figure 2 Detailed micrographs of bone defects grafted with *A*, coagulum; *B*, PRP mixed with particulated autogenous bone; *C*, PRP; and *D*, particulated autogenous bone after 1 week. *A* and *B*, Note the outgrowth of the woven bone trabeculae from the bone defect walls, and *B*, the bridging of the woven bone between the autogenous bone particles (AB) and the defect walls. *C*, PRP is highly concentrated in the center of the defect in the PRP group only. *D*, A typical micrograph illustrating woven bone (WB) formation. The mineralized bone matrix is covered by osteoid seams lined by a continuous layer of osteoblasts (*arrows*). BV = blood vessels; OB = old bone belonging to the defect walls.

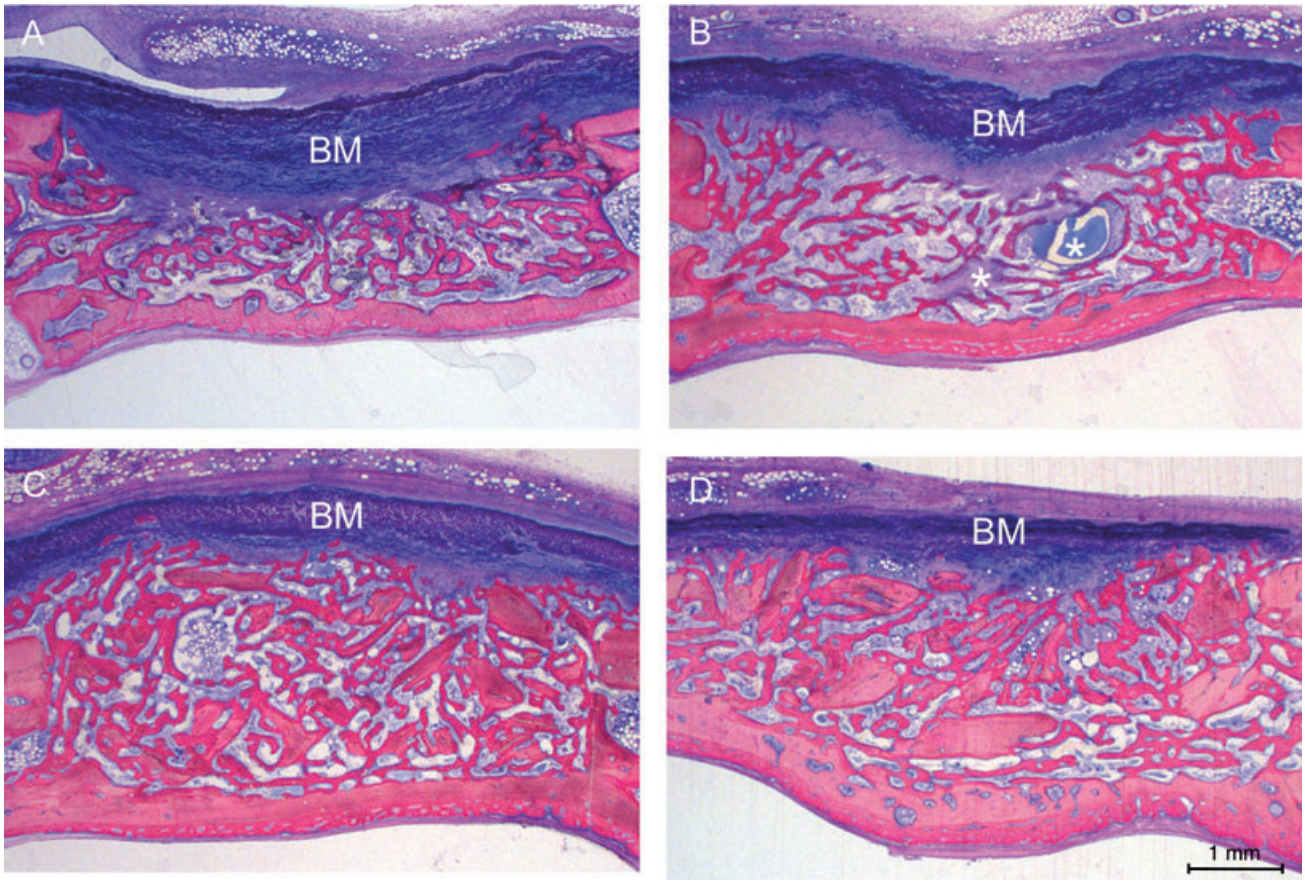

Figure 3 Overview micrographs of defects grafted with *A*, coagulum; *B*, PRP; *C*, particulated autogenous bone; and *D*, PRP mixed with particulated autogenous bone after 2 weeks. Each bone defect is lined by three bone walls (one at the defect base and two lateral to the defect) and a collagen barrier membrane (BM). The collagen membranes in the coagulum *A*, and PRP *B*, groups are slightly concave, whereas those in the autogenous bone group without *C*, and with PRP *D*, are slightly convex or flush, respectively. For all treatment modalities, newly formed bone had bridged the entire defect area under the collagen membrane with a scaffold of woven bone. *B*, A residual PRP concentrate (*asterisks*) is still visible in the PRP group.

in the active bone formation process occurring throughout the defect area (Figure 4, A). For all treatment groups, fibrous immature (primary) bone marrow filled the intertrabecular space. A small residual core of PRP was present at the center of the PRP group (Figure 3, B). Peripheral to this PRP accumulation, the woven bone appeared less organized than in the remaining defect area, and the marrow space appeared more cellular than in the coagulum group (Figure 3, A). In contrast to the PRP group (Figure 3, B), most of the PRP was no longer apparent in the autogenous bone + PRP group (Figure 3, D). For both the autogenous bone group without (Figure 3, C) and with PRP (Figure 3, D), more osteoclasts were present on the autogenous bone particles than at 1 week (Figure 4, B). Furthermore, within the autogenous bone only group, a portion of the newly formed bone consisted of more mature parallel-fibered bone than observed for all other groups (Figure 4, C).

Four Weeks. For all treatment groups (coagulum = Fig. 5, A; PRP = Figure 5, B; autogenous bone = Figure 5, C; autogenous bone + PRP = Figure 5, D), the bone density had increased (Figure 6, A). The trabeculae had almost doubled their width by apposition of denser, parallel-fibered bone. The presence of an osteoid seam lined by osteoblasts was indicative of ongoing bone apposition (Figure 6, B). The bone marrow had matured, as indicated by an increased number of adipocytes interspersed among islands of blood-forming cells (Figure 6, B). The corticalization of the lamina externa was not yet complete. With regard to the autogenous bone group without (Figure 5, C) and with PRP (Figure 5, D), the bone graft particles were well

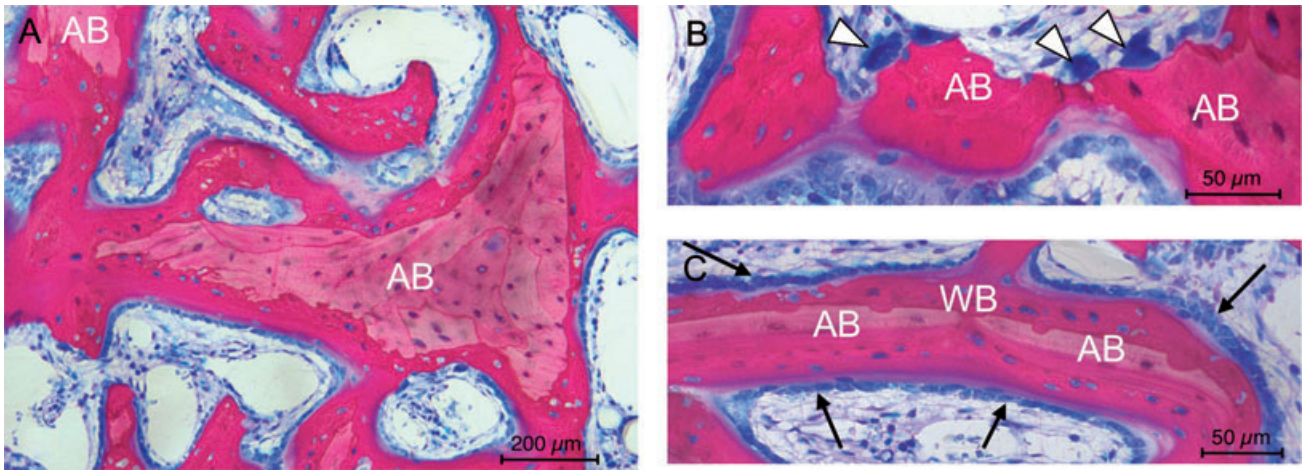

Figure 4 Detailed micrographs of bone defects grafted with particulated autogenous bone after 2 weeks. *A*, All autogenous bone particles (AB) are covered with new bone. *B*, Osteoclasts (*arrowheads*) are seen on the autogenous bone particles. *C*, The autogenous bone alone group showed the most mature bone formation, as indicated by the presence of parallel-fibered bone that was deposited onto woven bone (WB). An osteoid seam and osteoblasts (*arrows*) indicate ongoing bone formation.

integrated within the newly formed bone trabeculae and their size was decreased as a result of osteoclastic resorption.

Histomorphometry

No statistically significant differences in total new bone formation were detected among the four treatment types at either 1, 2, or 4 weeks. Interestingly, there was significantly greater percentage of lamellar bone than woven bone at 2 weeks for *only* the autogenous bone group. All other treatment groups (coagulum, PRP, and A-PRP) resulted in relatively equal amounts of woven and lamellar bone (Figure 7; Table 3). After 4 weeks, all groups demonstrated a statistically

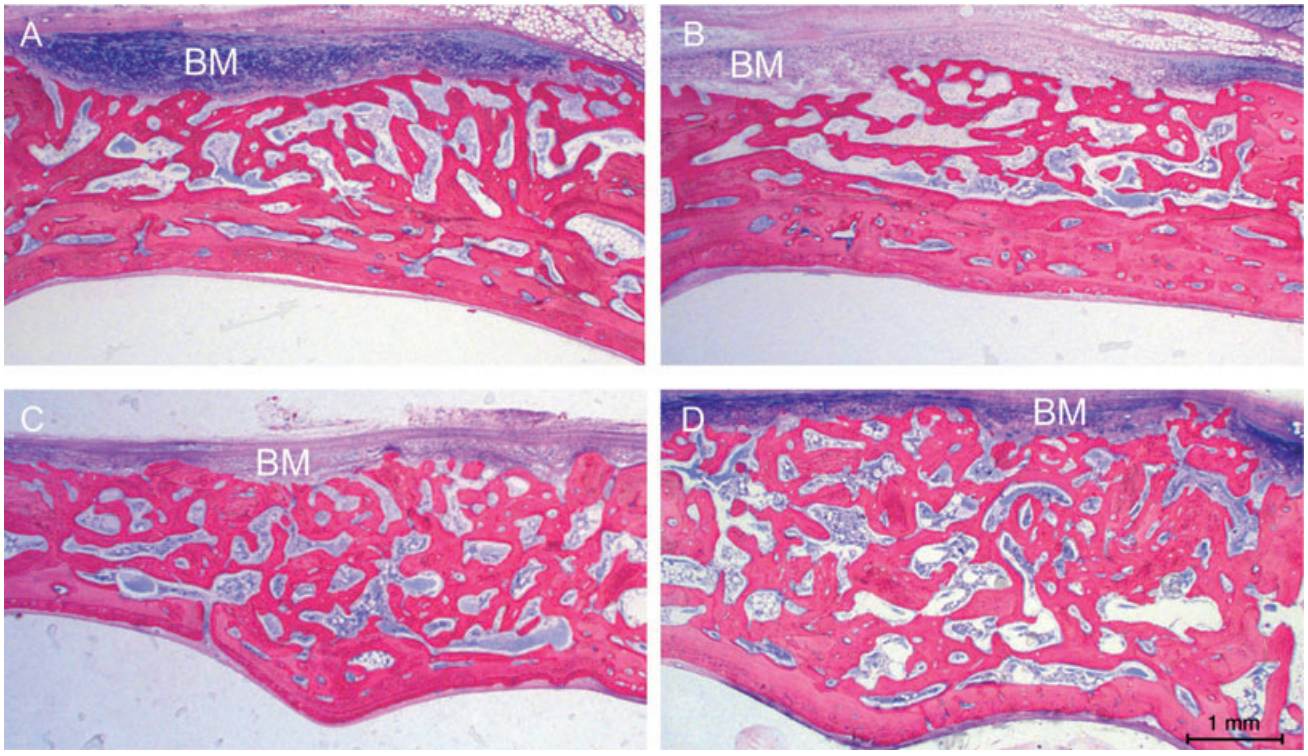

Figure 5 Overview micrographs of defects grafted with *A*, coagulum; *B*, PRP; *C*, particulated autogenous bone; and *D*, PRP mixed with particulated autogenous bone after 4 weeks. For all treatment groups, the bone density in the defect area under the collagen barrier membrane (BM) had increased over time.

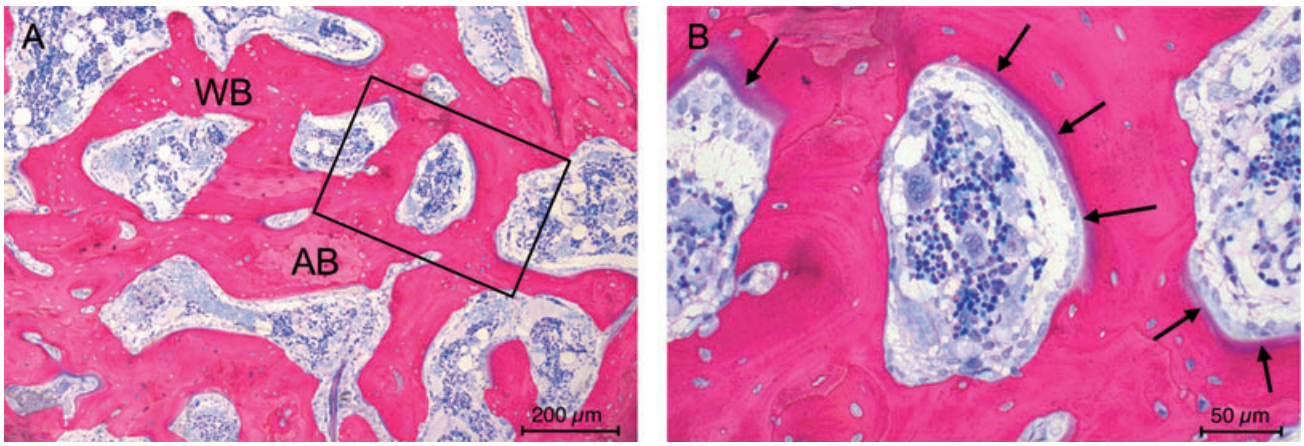

Figure 6 Detailed micrographs of bone defects grafted with particulated autogenous bone after 4 weeks. The rectangle shown in *A* is enlarged in *B*. *A*, Thick bone trabeculae consisting of a core of autogenous bone particles (AB) or woven bone (WB) are covered with dense, parallel-fibered bone. *B*, The presence of an osteoid seam lined by osteoblasts (*arrows*) is indicative of ongoing bone formation. An increased number of adipocytes interspersed among islands of blood-forming cells indicates maturation of the bone marrow.

significant higher component of lamellar bone over woven bone.

The percentage of residual graft was significantly reduced for each consecutive time point for both defect types containing autogenous bone (A-PRP, $p = .0047$; and A, $p = .0166$), indicating constant graft resorption over time. There were, however, no significant differences in residual graft between A and A-PRP at any of the three different time periods.

DISCUSSION

Growth factors, though theoretically appealing for their potential to minimize donor site morbidity and provide predictable bone regeneration, have a complex

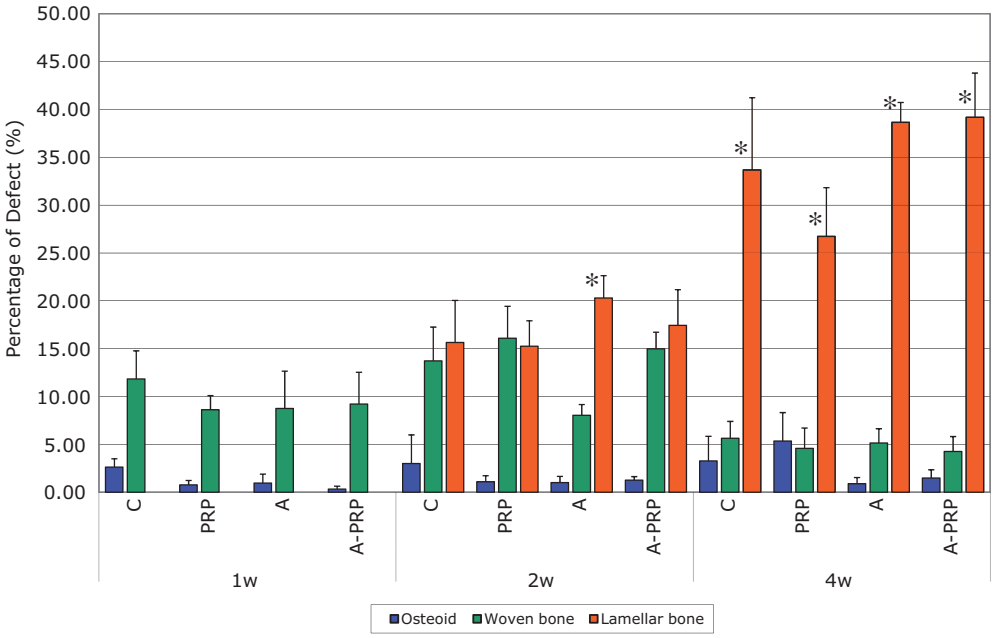

Figure 7 Effect of treatment type (graft) on the percentage of new bone formation. Histomorphometric quantitation of osteoid, woven bone, and lamellar bone for each defect treatment at different healing periods (1 w = 1 week; 2 w = 2 weeks; 4 w = 4 weeks): C = coagulum control; PRP = platelet-rich plasma (PRP); A = autogenous bone; A-PRP = autogenous bone + PRP. Results were then used to calculate the group mean (±SEM; $n = 5$). *Significant differences between woven and lamellar bone formation ($p < .05$).

TABLE 3 Effects of PRP, Autogenous Bone, and Their Combination on New Bone Formation

	Osteoid	Woven Bone	Lamellar Bone	Total New Bone Formation
1 week				
Coagulum	2.63 ± 0.88*	11.84 ± 2.94	0 ± 0	14.47 ± 2.34
PRP	0.77 ± 0.47	8.61 ± 1.49	0 ± 0	9.37 ± 1.41
Autogenous bone	0.95 ± 0.95	8.75 ± 3.91	0 ± 0	9.70 ± 4.85
Autogenous bone-PRP	0.32 ± 0.32	9.21 ± 3.34	0 ± 0	9.53 ± 3.21
2 weeks				
Coagulum	3.00 ± 3.00	13.72 ± 3.54	15.65 ± 4.40	32.37 ± 7.05
PRP	1.09 ± 0.64	16.09 ± 3.34	15.26 ± 2.66	32.44 ± 1.37
Autogenous bone	1.01 ± 0.66	8.03 ± 1.14	20.29 ± 2.35†	29.33 ± 2.11
Autogenous bone-PRP	1.26 ± 0.38	14.97 ± 1.76	17.43 ± 3.74	33.66 ± 3.53
4 weeks				
Coagulum	3.26 ± 2.59	5.64 ± 1.77	33.68 ± 7.55†	42.58 ± 8.68
PRP	5.35 ± 2.98	4.58 ± 2.14	26.72 ± 5.11†	35.23 ± 7.06
Autogenous bone	0.89 ± 0.66	5.14 ± 1.50	38.65 ± 2.08†	44.68 ± 3.67
Autogenous bone-PRP	1.48 ± 0.87	4.25 ± 1.58	39.17 ± 4.63†	44.68 ± 5.40

*Results presented as the mean + SEM.
†Significantly greater ($p < .05$) than the percentage of woven bone.
PRP = platelet-rich plasma.

mechanism of action. Each factor has a different effect on signal transduction of bone formation and matrix mineralization. The interaction with one another may trigger certain cascades of signaling proteins and pathways, leading to gene expression followed by protein production. The exerted outcome may even be inhibitory, depending on the effective composition and respective concentrations, or if used continuously rather than in a pulse fashion.[19] This particular aspect of PRP, consisting of multiple autologous growth factors already involved in the native healing response and rendering its effect on multiple pathways, differentiates PRP from single recombinant growth factors that direct their effect on single signal transduction and thus its appeal. Indeed in previous studies, PRP induced significantly greater new bone formation when combined with autogenous bone,[4,20,21] or alone[21] as compared to a coagulum-filled control.

Within the confines of the current study, PRP did not exert an additional effect on coagulum or autogenous bone. The results concur with studies that demonstrated no additive effect in bone regeneration procedures with any of the various types of graft material: autogenous bone,[22,23] human allograft,[12] xenograft,[24] or an alloplast.[11] It has been suggested that platelet concentration plays a role in its effect in bone regeneration[25]: at low concentrations, the effect is suboptimal, while higher concentrations might have a paradoxically inhibitory effect. It has been hypothesized that only a limited range of platelet concentration of approximately 1,000,000/μL has advantageous biological effects. While there was a lack of a positive effect in the current study whose mean platelet concentration was 1,761,930 ± 680,200/μL, there was no inhibitory effect.

Perhaps the greatest difficulty of the PRP technique is inconsistency of the final preparation, reflected by wide individual variability and the impracticality of intraoperative laboratory analysis for additional adjustments of platelet concentration. In spite of extensive measures to standardize the final PRP preparation in the current study, a rather large range of platelet concentration and growth factor concentration was found. The basal range of platelets found in whole blood was 138,000–619,000/μL. The final concentration of platelets in PRP did not translate proportionally. For example, an individual animal with a basal level of platelets, 138,000/μL, yielded a concentrate 13.74 times for a final platelet concentration in PRP of 1,896,000/μL. In contrast, another individual animal whose basal level of platelets was 447,000/μL yielded a concentrate of "only" 2.62 times whole blood with the very same standard procedure. Additionally, PDGF-AB and TGF-beta1 concentrations were found to be quite variable. Indeed, only

TGF-beta1 and platelet concentration in PRP were found to be significantly positively correlated. This difficulty of efficient standardization poses limited application of PRP. In a clinical setting, intraoperative measures to control the exact concentrations would be highly unrealistic and lead to increased operative time.

A commonly cited critical size defect in the rabbit calvarium has been established to be a 15-mm diameter bicortical defect at 24 weeks[26] which, however, would require one animal per defect. In the current study, the selected defect diameter was not intended to be a critical size defect for the study's longest healing period of 4 weeks, but instead was selected to allow the analysis of all four treatment types within the same animal. Furthermore, a monocortical defect was chosen to avoid possible confounding effects of brain herniation and edema which commonly occurs in bicortical defects.[27] The scope of the present study was not to prove the absolute efficacy of PRP in critical size defects but instead study its effect in the rate of early bone formation related to baseline (coagulum defects) and compared with the gold standard graft material (autogenous bone). Under the limits of the chosen model, differences in total new bone formation among treatment groups could not be discriminated. In spite of the model's limitations, it should be noted that autogenous bone alone attained a significantly higher percentage of lamellar bone than woven bone at 2 weeks, suggesting a more mature new bone composition. In contrast, PRP, whether alone or mixed with autogenous bone, resulted in relatively equal amounts of woven and lamellar bone, as seen for the coagulum-filled control defects. At 4 weeks, all groups reached the same level of bone remodeling, with lamellar bone being the predominant new bone type. One may argue that with unsupported, "soft" defects, such as with coagulum or PRP alone, a collapse of the membrane would occur and results could not be compared with "supported" defects filled with a hard filler, such as autogenous bone with or without PRP. In the current study, this was not a confounding factor; no statistical difference was observed between membrane- or cortex-expressed data.

Although its theoretical use is supported by basic science studies,[28] standardization and widespread clinical application remain in question. Within the limits of the selected model, we conclude that: (1) in early healing, PRP alone or when added to autogenous bone, does not result in a more advanced level of bone remodeling as compared to coagulum, (2) autogenous bone alone generates more highly remodelled bone earlier than autogenous bone combined with PRP, and (3) PRP has no apparent effect on autogenous graft resorption. Further investigation is required to determine whether PRP significantly exerts a positive influence in repair-impaired bone, such as with diabetes, osteoporosis, hyperparathyroidism, or irradiated bone in cancer patients.

ACKNOWLEDGMENTS

The authors would like to thank Prof. R. Schenk, Prof. W. Wagner, and Dr. S. Jensen for their input; Mrs. B. Hoffmann, Mr. D. Reist, and Ms. S. Owusu for specimen preparation; Ms. C. Moser for her surgical assistance; PD Dr. G. Hafner and Mrs. R. Schweigert for the growth factor analysis at the Institute of Clinical Chemistry and Laboratory Medicine, under the direction of Dr. Gernot Weibrich, Departments of Prosthetic Dentistry and Oral and Maxillofacial Surgery, all at Johannes Gutenberg University, Mainz, Germany; PD Dr. P. Manil, Dr. A. Wetterwald, Ms. I. Tschudi, Mr. M. Siegrist, Ms. Kapfinger, Ms. V. Gaschen, and Ms. V. Winkelmann for their participation in preliminary studies; Dr. D. Mettler, Ms. O. Beslac, and Mr. D. Zalokar at the surgical research unit (ESI), University of Berne for the animal and anesthesia management; Prof. Dr. B. Lämmle, Dr. Med. M. Solenthaler, and Mrs C. Jeanneret at the Division of Hematology, Inselspital, Berne, Switzerland for the analysis of whole blood, PRP, and PPP; and Prof. A. Lussi for the preliminary statistical analysis. Barrier membranes and PRP kits were donated by Geistlich, Wolhusen, Switzerland and Harvest Technologies Corporation, Plymouth, MA, USA, respectively. None of the authors has financial interests in the investigated biomaterials. The study was supported by a grant from the ITI Foundation for the Promotion of Oral Implantology, Basel, Switzerland (grant no. 295-03) and a grant from the Swiss National Fund (grant no. 32003B-100404).

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